

A Potential Hazard: Explosive Production of Mutations by Induction of Mutators*

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Introduction

A systematic study of the genes that control mutation rates began about five years ago both in prokaryotic and eukaryotic organisms. Before this, mutator mutants generally were regarded as freaks, causing their mutagenicity possibly by the build-up of a product of intermediary metabolism which was a mutagen. The concept of differences in the spontaneous mutation rate as a natural phenomenon was suggested by Ives (1) in 1950, but it was not until 1962 that mutation rate enhancement was recognized as a natural component of the meiotic process by Magni and von Borstel (2).

In 1966, defective DNA polymerase was noted to have mutator activity (3), and in the following two years defective enzymes associated with DNA repair were implicated in a large variety of organisms (4). Still, the isolation and identification of mutator mutants were difficult at best, although Liberfarb and Bryson (5) were able to isolate a number in *E. coli*. Cox and his collaborators (6) have been studying these and other mutators in *E. coli*, and Drake (7) has been examining likely candidates for mutator activity among genes encoding known biochemical functions in the bacteriophage T4. Mutator mutants were induced and selected in the yeast *Saccharomyces* (8). The unex-

pected and impressive result was the very high frequency with which strains with altered mutation rate were found. With ethyl methanesulfonate as the mutagen, the frequency of induced mutator mutants in yeast was approximately one-tenth of the frequency of induced auxotrophs. A sensitive method for measurement of spontaneous mutation rates is available with which 2- or 3-fold increases in mutation rate are commonly detected, but strains with 20- to 30-fold enhancements in the spontaneous mutation rate are also frequently seen.

Antimutator mutants, which reduce the spontaneous mutation rate have been described, but we have barely begun to study these (9,10). The relative frequency with which mutator and antimutator activities are induced is not known, although it is recognized that it will be important to look into this in the near future.

The Hazard of Induction of Mutator Mutants

In a discussion about two years ago, W. L. Russell pointed out that if mutator mutants are induced with any enhanced frequency whatsoever, these could be potentially more hazardous to a population than induction of the standard sublethal and lethal defects.

The problems involved in the detection of changes in mutation frequencies in human populations have been discussed by Neel (11). We shall concern ourselves with the

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potential hazard to populations of the induction of mutators.

Leigh (12) has considered the fate of mutator genes which affect equally the rates of production of both "forward" and "backward" mutations at another locus in haploid populations. Where a difference in selective value exists between the two alleles at the latter locus, he found that natural selection in the long run will favor the lowest possible mutation rate in sexual populations. In a constant environment, the selective disadvantage of the mutator, say on a different chromosome from that of the locus it affects, will be $2u's$ (where u' is the increased mutation rate caused by the mutator, and s is the selective disadvantage of the mutants induced by the mutator). Even under conditions of a regularly changing environment of periodicity long compared to the time of allele substitution, such a mutator will be selected against in the long run. Given the size and migration patterns of populations comprising most of our species, it is doubtful that group selection could any longer be considered a realistic mechanism for bringing about "optimum" mutation rates which would be greater than the physiological minimum [see Leigh (12) for additional discussion], but this does not preclude the possibility of considerable variation among isolated populations, given certain conditions, in the frequencies of mutator activity.

For a general mutator the selective disadvantage can be obtained by summing its selective disadvantages at all loci which it affects. Given that the prior probability of synteny between two autosomal loci in man is about $1/18.5$ (13), we may consider as a first approximation that no more than 5% of the loci which are affected by a given general mutator can be absolutely linked to that mutator. Each of this minority of loci contributes to the selection coefficient of the mutator the augmentation of its mutation rate, u'_i in each generation. The majority of loci (approximately 95%) affected by the mutator are asyntenic, and each adds $2u_i s_i$ to the total selective disadvantage of

the mutator. Clearly, a mutator would have to affect the larger portion of the genome each generation for natural selection to control its spread under this model.

However, if these selective values are small enough, then we anticipate diversity among human populations with respect to the frequencies of specific mutators and antimutators due to the effects of random genetic drift. This diversity may have been enhanced by the population structure of the human species during most of its evolution as inferred from studies of contemporary primitive populations (14). Appreciable frequencies of a single deleterious recessive allele can be attained in populations which now may be large, but which evolved in isolation from a small group of founders with an early phase of rapid population growth (15).

Thus the possibility of polymorphic frequencies of both mutators and antimutators would confound attempts to detect differences in induced mutation frequencies in human populations. Interpretation of the cause(s) of any differences in mutation frequencies between populations, perhaps including human races, be they statistically significant or not, might be more readily attributable to mutator activity than to mutagens in the environment.

Population Dynamics of a General Mutator

We may consider the population dynamics of a single general mutator in a finite population as being the same as that set out by Li and Nei (16) for a single deleterious mutation assuming that there is no further mutation of the same type. With constant population size and selective disadvantage, should the mutation be completely recessive with respect to fitness, the total number of individuals affected by such a deleterious mutant, that is, the number of mutant homozygotes, is relatively small. However, the expected total numbers of mutant heterozygotes and the average time to extinction of this allele increase as the effective population size increases. On the other hand, for a single

deleterious mutant which is partially recessive with respect to fitness, the total number of affected individuals and the average extinction time are almost independent of the effective population size.

Should the population be increasing in size at the time of appearance of the deleterious gene—and the growth rate of the human species as a whole appears to have increased since at least the industrial revolution—the total number of individuals affected by a single mutation will be augmented, and the average extinction time lengthened. Furthermore, Li and Nei note that the assumption of a constant intensity of selection against a deleterious mutation is not likely to be met in human populations where environments are changing and especially where medical treatment of genetic diseases progresses. The theory and calculations provided by Li and Nei imply that the resulting cost to human society in terms of genetic damage which a general mutator would create could be tremendous.

Some Thoughts on Mutators and Their Detection in Human Populations

There is not one mechanism that has sufficed to explain malignant growth. We consider the possibility that mutators might be responsible for abnormal cell replication or repair leading to malignancy. Is it possible, then, that pedigrees of inherited malignant disorders demonstrate the segregation of such a mutator?

Knudson (17) and Knudson and Strong (18) have demonstrated statistically a causation consistent with two mutational events for each of the following three cancers in man, retinoblastoma, neuroblastoma, and pheochromocytoma. In the nonhereditary form of any one of these types of cancer, both mutations occur in somatic cells. In the hereditary cases, predisposition is determined by an inherited (germinal) dominant mutation, and the second mutational event occurs in somatic cells. This latter event is evidently rare or, at least, its phenotypic manifestation is rare. Also, it appears that the

dominant germinal mutation is tumor-specific.

Knudson and Strong have calculated that among individuals who have inherited a first mutation, a second (somatic) mutation yields a number of tumors which averages about three for retinoblastoma, about one for neuroblastoma, and two or three for pheochromocytoma uncomplicated by other tumors. Their estimates of gene penetrance based on the zeroth term of the Poisson distribution are, therefore, 0.63 for neuroblastoma, 0.87–0.95 for pheochromocytoma, and 0.95 for retinoblastoma.

Although other interpretations are possible, the inheritance of mutators themselves may be reflected in familial variation of penetrance of such cancers, in heterogeneity of rates of discordance among twins, and in those cases where, for example, pheochromocytomas are additionally associated, perhaps uniquely (18), with other cancers and mutations.

It would be of especial interest to detect either enhanced or reduced penetrance of these types of cancer, because this would be clear evidence for either a difference in spontaneous mutation rates or a difference in the ability of the hosts to eliminate cancerous cells.

Furthermore, it is becoming more and more obvious that not only are mutagens carcinogenic, but that carcinogens are mutagenic (19). Thus if mutators can cause mutations, why can they not cause types of cancer in addition to those described above? Differences in rate of spontaneous tumors among populations may be the simplest way of detecting differences in spontaneous mutation rates. Mutators may be present wherever familial tendency toward a variety of cancers is observed.

Recommendations for Further Research

These considerations prompt several lines of research. First, the rates of induction of mutators and antimutators must be measured in a variety of cells, beginning with prokaryotes and the lower eukaryotes

where this can be most easily done. This would serve as an indicator of the magnitude of the problem for the human species. Also, one could analyze the frequency of induction of secondary mutators by the first mutator, a true explosive effect. Second, mutators must be induced and studied in mammalian cell lines; their rate of induction and their mutator properties, such as rate of malignant transformation, would be useful to evaluate their influence as a hazard to the human species. Third, the measurement of spontaneous mutation rates in cell lines from humans could be useful in determining the variation in the spontaneous mutation rate from person to person and population to population. Fourth, mutator phenotypes, like familial malignancies with variable penetrance or spontaneous tumorigenesis, should be vigorously sought.

Conclusions

Potentially the genetically most hazardous events that could result from exposure to environmental mutagens are the induction of mutators. An overall enhancement of spontaneous mutation rates would lead to the creation of deleterious mutations which could persist almost indefinitely in the expanding human species. The relative frequencies of induction of antimutators and mutators are not known. Nor do we as yet fully understand the mechanism(s) by which mutators enhance the induction of mutations. Furthermore, the spectra of activities of spontaneous and induced mutators need to be characterized in order to anticipate more adequately the societal burdens which would be caused by the resulting "explosions" of genetic damage.

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